Oligotrema psammites: a New Ascidian belonging to the Family Molgulidæ.

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With Plates 19—23.

The interesting little Ascidian which forms the subject of this paper was dredged by Dr. A. Willey from a depth of fifty fathoms off Lifu, New Britain, and was sent to me among a collection of Zoanthids made in the same locality. Externally the animal has so little resemblance to an Ascidian, and has so many features resembling those of Anthozoa, that it might well be mistaken for a member of the latter group. The body is sack-shaped and covered with grains of sand, like that of a Zoanthid. The mouth, or more correctly, the branchial aperture, is transversely elongated and bordered by tumid lips (Fig. 2), and there is a circlet of six pinnate arms or tentacles resembling those of an Alcyonarian surrounding the mouth. The internal organs can only be distinguished as a dark mass through the translucent walls of the body, and the atrial siphon is so small and inconspicuous that it might easily escape observation. My attention was at once attracted by the presence of six pinnate tentacles, and, suspecting that the animal was new to science, I cleared it for preliminary examination in dilute glycerine, which rendered the test transparent and exposed clearly the intestine, the atrial aperture, and the other structures shown in Fig. 1. Further examination left no doubt that the animal was an Ascidian.

As I was unwilling to destroy the single specimen, I attempted to work out its anatomy by dissection, and with this object in view I made a longitudinal incision in the test, as shown in Fig. 3, and afterwards cut through the body just below the circumoral arms in order to study the latter structures; but the tissues proved to be so brittle and fragile after long immersion in spirit that I abandoned any further attempt at dissection, and embedded the two parts into which the animal was divided in paraffin. A series of sections was cut and stained with borax carmine, followed by picro-nigrosin or picro-indigo-carmine. Though the epithelia were in many places detached or peeled off from the underlying tissues, the general histological preservation was good, and in most cases the cell elements, even of the fragmented epithelia, were remarkably well preserved. I have therefore been able to make a tolerably exhaustive study both of the anatomy and histology of the animal, but before going into details I will define the genus and species.

Family MOLGULIDÆ.

Oligotrema, nov. gen.—Body sack-shaped; the branchial and atrial apertures distant; the branchial aperture terminal, large, transversely elongated, surrounded by a circlet of six muscular, pinnate arms or tentacles. The atrial aperture minute, without lobes, placed on a small papilliform eminence of the test near the hinder end of the body on the dorsal side. The branchial sack much reduced and confined to the anterior third of the body.

Oligotrema psammites, n. sp.

With the characters of the genus. The test thin, but tough, translucent; its whole surface covered with hair-like processes, to which sand-grains are attached; these capilliform processes are moderately long on the outer (aboral) surfaces of the arms, short over the remainder of the anterior three-quarters of the body, long over the posterior quarter.

Length of the body (antero-posterior), 17·5 mm.; average diameter of the body, 8·75 mm.; measurement from tip to tip of the expanded arms, 10·25 mm.

Habitat—Lifu, New Britain, 50 fathoms. Only a single specimen was collected by Dr. Willey.

As I find it difficult, if not impossible, to give an intelligible account of the anatomy of Oligotrema while retaining the use of the term "mantle," and as the nomenclature of the layers of which the Tunicate body is composed is in a state of confusion in this and other respects, I must, before giving a detailed account of the structure of the species, enter into a short explanation of the morphological value of the different layers, and propose some new terms which, I hope, will tend to prevent obscurity.

The different coats or layers of the Tunicate body are generally described as follows, beginning from the outside:—(1) The test (it has also been called the external tunic), which is essentially a cuticular structure, much modified by the presence of abundant intrusive mesoderm elements. Its composition and relations are well understood, and require no further mention in this place. (2) The mantle (also called the inner tunic, body-wall, etc.). This sheet of tissue, encircling the whole body, is covered on its outer surface by a layer of epithelial cells, which is the external ectoderm. Its internal surface is lined by an epithelial layer, also of ectodermal origin, the atrial epithelium; and between these two there is a mass of tissue, consisting of a form of connective tissue permeated by blood-spaces and sinuses, and containing circular and longitudinal muscle-fibres, the gonads, the renal organ, heart, stomach, intestine, etc., embedded in it. The so-called mantle is separated by a considerable space, the peribranchial or atrial chamber, from (3) the branchial sac, the wall of which is made up as follows:—Externally there is the atrial epithelium; inside this there is a layer, for the most part thin, of connective tissue channelled by blood-spaces; within this, again, and lining the cavity of the branchial sac, is the endodermal epithelium. The walls of the branchial sac are, in most simple Ascidians, so extensively pierced by the branchial slits or stigmata that the arrangement of their component layers is not obvious, but in Oligotrema the branchial sac and the number of the stigmata are so much reduced that the relations are very clearly seen in sections. The arrangements just described are, of course, well understood, and have been particularly clearly explained by Yves Delage, and Herouard in the eighth volume of the 'Zoologie Concrète;' but these authors, while showing a true appreciation of the morphology of Tunicates in other respects, seem to me to make a grave mistake in describing the complex of blood-spaces in the connective-tissue layers of the "mantle" and branchial sac as a "schizocœle." A schizocœle, as defined by the authors of the name, is a form of cœlomic cavity distinguished by its mode of development from an enterocœle. The blood-sinuses in the "mantle" and branchial sac are not cœlomic spaces, but contain blood, and are appropriately classed under Professor Lankester's term hæmocœle. Moreover we know that, in some simple Ascidians at any rate, the cœlom is developed as an enterocœle from outgrowths of the primitive gut, but is obliterated early in larval life by the conversion of its walls into mesenchyme, which fills up the space not occupied by the nervous system and notochord, between the ectoderm and endoderm. The term schizocœle, therefore, is doubly unfortunate.

Equally unfortunate, I think, is the term "mantle" applied to the outer layers of the body-wall. It suggests, and was originally intended to suggest, a genetic relationship with the mantle of Mollusca, but this view has long since been abandoned, and the name has now only the sanction of long usage to recommend it. Furthermore it is misleading, for it implies an external coat formed as an outgrowth from the body-wall, whereas the so-called mantle is in fact the body-wall, and contains most of the important viscera, as well as the musculature and the nerve-ganglion. It may most correctly be described as the external part of the body-wall, which has been, as it were, accidentally cut off from the internal part surrounding the pharynx (and in Oligotrema the œsophagus) by the ingrowth of the atrial and peribranchial cavities.

In the light of our existing knowledge of development and comparative anatomy, a Tunicate must be regarded as an animal in which the primitive cœlom has been obliterated and its place taken by a mass of connective tissue permeated by an irregular system of blood-spaces and sinuses (hæmocœle). In the adult the cavities of the gonads, and possibly the pericardial cavity[1] and the lumen of the renal organ, are the only representatives of the original cœlom. Considered from this point of view the Tunicate is a solid animal,—that is to say, the space between the gut and the external epithelium is theoretically filled up with mesodermic tissue, except for the relatively small cavities of the gonads, the pericardium, and renal organ. To this mesodermic tissue I propose to give the name pleromе, from πλήρωμα, a thing filled up.[2]

If we imagine the formation of the atrial cavity to be delayed to a much later period of development than is actually the case, a transverse section taken midway through the body of an Ascidian, before the establishment of the atrial cavity, would have the characters shown in the annexed diagram, Fig. 1. The area shaded with dots represents the plerome. The atrial cavity would be formed from two dorso-lateral invaginations of the ectoderm, which would become confluent in the dorsal region and give rise to a flattened sac, overlying the dorsal aspect of the pharynx and opening to the exterior by the atrial aperture. The ventro-lateral horns of this sac would push their way downwards right and left of the gut to form the right and left peribranchial cavities, and in so doing would divide the plerome into an inner moiety surrounding the gut, which we may call the endoplerome, and an outer moiety forming with the external ectoderm the wall of the body. This latter moiety is what is usually called the mantle; I propose to call it the ectoplerome. It contains the gonads, renal organ, heart, intestine, and other viscera. On the formation of the branchial slits the endoplerome of the branchial sac is, of course, broken up, and is represented by the mesoblast of the branchial bars, as indicated in the diagram in the text, Fig. 2. In most Ascidians, owing to the great size of the branchial sac, the endoplerome is practically reduced to the blood-

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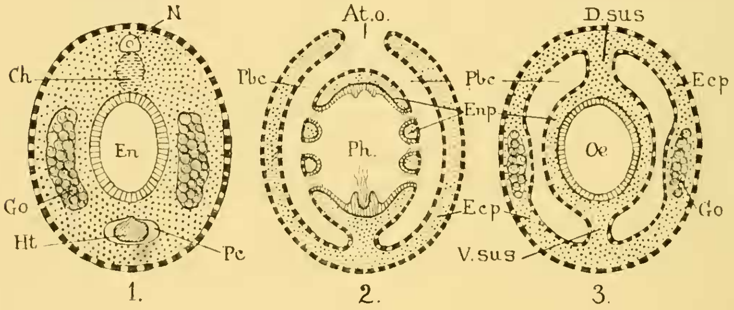
[1] The pericardial cavity of Ascidians, being formed from the ventral wall of the pharynx, may possibly be regarded as an independently developed portion of the enterocœle.

It may be objected that this term is already used by botanists. But its botanical connotation is so different from that now proposed, that it is unlikely that any inconvenience can arise.



1.—Section of a hypothetical Tunicate larva before the formation of the atrial and peribranchial cavities. The mesoderm (plerome) is a solid mass of tissue in which the gonads and the heart enclosed in its pericardium are embedded.

2.—Diagrammatic transverse section through the pharynx of an Ascidian showing the plerome divided into an outer layer (the ectoplerome) and an inner layer (the endoplerome), surrounding the pharynx.

3.—Diagram of a section through the œsophagus of Oligotrema psammites. *At. o.* Atrial aperture. *Ch.* Notochord. *D. sus.* Dorsal suspensory fold. *Ecp.* Ectoplerome. *Enp.* Endoplerome. *En.* Enteron. *Go.* Gonads. *Ht.* Heart. *N.* Nerve-cord. *Oe.* Œsophagus. *Pbc.* Peribranchial cavities. *Pc.* Pericardium. *Ph.* Pharynx. *V. sus.* Ventral suspensory fold. The ectoderm is represented by a thick broken line; the plerome (mesoderm) is shaded with dots.

sinuses in the branchial meshwork; but in Oligotrema, in which the branchial sac is very small, and the œsophagus and stomach are relatively larger and occupy the posterior two-thirds of the body, the endoplerome is a well-defined, continuous sheet of tissue surrounding the œsophagus and anterior portion of the stomach. As the peribranchial cavities do not meet in the mid-ventral line, the branchial sac, and in Oligotrema also, the œsophagus and stomach, are attached to the ventral body-wall by a partition consisting of a sheet of plerome covered on both sides by atrial epithelium. This is the ventral or sub-endostylar suspensory fold. There is a similar dorsal suspensory fold separating such prolongations of the right and left peribranchial cavities as extend beyond the limits of the cloaca. The relations of these folds to the ecto- and endoplerome are shown in the diagram in the text, Fig. 3. A justification for this somewhat lengthy discussion of facts already well known will be found, I hope, in the practical utility of the terms I have proposed. I will now give a detailed description of the anatomy and histology of Oligotrema.

The Test.

The external appearance of the test has been mentioned in the description of the species. It is relatively thin over the greater part of the body, but is locally thickened in the neighbourhood of the branchial and atrial apertures and on the external aspects of the proximal moieties of the arms. It is involuted to a considerable extent, both at the branchial and atrial apertures. The branchial involution forming the branchial siphon is thick, and its free surface is thrown into a number of longitudinal folds. The atrial involution of the test is thin, but extends for some distance inwards (fig. 34). The substance of the test is homogeneous, or in some places shows traces of fibrillation, and gives the cellulose reaction with sulphuric acid and iodine. It contains numerous immigrant corpuscles (amœbocytes), which are nowhere swollen up to form the large vesicular cells common in the tests of simple

Ascidians. The amœbocytes of the test are generally elongate-oval in form, sometimes irregular and amœboid in shape; each contains a small, deeply staining nucleus, situated to one side of the cell; the cytoplasm is vacuolated, and in most cases contains granules of various forms. In some cases the granules are of a bright yellow colour, and these will be described more particularly further on. The hair-like processes or villi of the test do not differ from those described in many other Molgulidæ and in some Cynthiadæ. The villi are longest and most numerous on the rounded posterior (aboral) end of the body; they are almost equally abundant, but shorter and thicker, on the outer faces of the arms; they are short and more sparsely distributed over the rest of the body. The villi are hollow, and most of them contain amœbocytes; some of them, especially the longer ones at the posterior end of the body, end in enlargements, to which grains of sand are fixed, like those of Molgula (Anurella) roscovita figured by de Lacaze Duthiers. The villi of the arms and body, however, have bluntly pointed or rounded extremities, and sand-grains, sponge spicules, etc., are attached to their sides by a flocculent yellow material, which, as I shall show presently, is probably excreted by some of the amœbocytes of the test. The substance of the test is penetrated in all parts of the body by somewhat sparsely distributed, knobbed, and tassel-like vascular processes of the body-wall. These will be more conveniently described in connection with the ectoderm, but it may be mentioned that they do not penetrate, or, as far as I could determine, enter into any relations with the hair-like villi of the test.

The Ectoderm.

The relations of the external ectodermal epithelium to the test could not be very satisfactorily studied owing to the shrinkage of the tissues of the body-wall and their separation from the test. In some places the epithelium was adherent to the test; in others it remained adherent to the body-wall. In both cases it could be seen to consist of a single layer of somewhat flattened, fusiform cells, whose pointed extremities interdigitate. These cells are easily recognisable because of the numerous minute granules of black pigment scattered through their cytoplasm (fig. 29, *ect*). Over the greater part of the surface of the body the ectodermal epithelium appears to be simply a flat layer applied to the internal surface of the test, but in the neighbourhood of the bases of the arms and the branchial siphon the relations between test and epithelium are more intimate. In these regions the body-wall is produced into irregular branching processes, covered with pigmented ectoderm, which penetrate into the softer and apparently semi-fluid internal layer of the test, as is shown in fig. 29. (In the figure the processes in question are separated from the test by shrinkage due to preservation in spirit.) In many of the pigmented ectoderm-cells there are yellow granules which are apparently formed in the ectoderm-cells themselves, for I could not find any traces of similar granules in the amœbocytes of the plerome in this region. But the amœbocytes which are passing through the ectoderm or have passed through it into the test are loaded with yellow granules (fig. 29, *amc*). It seems that the amœbocytes—one of them is shown clearly at *amc*—pick up the yellow granules formed by the ectoderm, and when loaded with them migrate into the test, pass through it, and discharge their contents on its free surface, thus forming the sticky, flocculent deposit which attaches grains of sand, sponge spicules, and other foreign substances to the test. Similarly amœbocytes laden with granules pass down the lumina of the hollow villous processes of the test.

The pigmented external ectoderm also enters into the composition of a peculiar form of tissue found on the ventral sides of the bases of the arms. The diagram, fig. 34, shows that near the bases of the arms there are recesses or indentations of the body-wall, and that the space between test and body-wall is prolonged some way into the body. These recesses are, of course, lined by ectodermal epithelium, and at their inner ends the epithelium is prolonged into a number of ramified ingrowths, which penetrate deeply into the plerome in much the same manner as the allantoic villi penetrate into the trophoblast of a mammal. Fig. 25 is an enlarged drawing of the area indicated by the reference number 25 in fig. 6. It is not sufficiently magnified to show details, but it exhibits the general relations of the tissues of this region, showing the powerful muscular bands of the sphincter, the longitudinal muscle-bands, *l. mus.*, inside of these bordering on the cavity *x*, and numerous well-defined blood-spaces, *bl.* The intrusive ectodermic ingrowths, *ec. i.*, are distinguished by their numerous dark nuclei, and the drawing gives a good idea of their number and extent, and of the relations they bear to the other tissues. Fig. 30 is a highly magnified drawing of a portion of fig. 25, showing the ectodermal ingrowths, *ec. i.*, formed of the characteristic pigmented ectoderm-cells, many of which contain yellow granules. The ingrowths penetrate into the blood-spaces, and there can be little doubt that the whole structure is subservient to the nutrition of the test.

The two tubes marked *vp.* in the same figure, whose walls are composed of one or more layers of cells with deeply stained nuclei, are cross-sections of two of the tassel-like vascular processes described above as penetrating the substance of the test in all parts of the body.

The general appearance of these vascular processes is best shown in fig. 5, *vp.* They are most abundant on the arms, but occur over the whole surface of the body, and, as the body-wall has shrunk away from the test, some of them have been pulled out of the crypts into which they fitted and remain adherent to the body-wall; others have been torn away, and remain embedded in the test. They vary very much in length; those on the arms are quite short, as shown in fig. 5, but in other parts of the body they may be of considerable length, penetrating for a long way into the substance of the test. Each is a simple tubular outgrowth containing a cavity, which on the one hand communicates with the blood-spaces of the plerome, on the other hand ends blindly at the knobbed distal extremity of the process. The single or double layer of epithelial cells composing their walls is continuous with the external ectoderm, but differs from the latter in not containing pigment granules. I was at first inclined to regard these processes as sense organs, but as I have been unable to find any nerve-fibres in connection with them or to distinguish any sense-cells on them, I must conclude that they are simply vascular processes, serving partly to attach the body-wall to the test, partly to aid in the nutrition of the latter.

The characteristic pigmented ectodermal epithelium also forms a lining to certain remarkable cavities which lie right and left of the branchial sphincter muscle. The relations of these cavities, which may possibly be artefacts due to contraction in spirit, will be described further on.

The atrial epithelium differs considerably from the external ectoderm, consisting of a pavement epithelium composed of very much flattened polygonal cells, devoid of pigment granules, and fitting closely to one another by their edges. The transition from the pigmented external epithelium, involuted along with the test into the atrial siphon, to the unpigmented atrial epithelium is abrupt. In the neighbourhood of the anus the atrial epithelium is modified. There is a tract of columnar, flagellated cells leading from the anus to the atrial siphon. These cells are very similar in shape to the curious flagellated cells forming the lining epithelium of the branchial sac, which will be described in connection with that organ.

The Plerome.

As described above, the body of Oligotrema must be thought of as if there had been a solid mass of tissue (the plerome) between the external ectoderm and the gut. Into this mass the atrial cavity has insinuated itself and, spreading right and left of the gut and branchial sac, has divided the plerome into an outer layer, the ectoplerome, corresponding to the "mantle" of previous authors, and an inner layer, the endoplerome, surrounding the gut. The inner surface of the ectoplerome and the outer surface of the endoplerome are, of course, covered by atrial epithelium. As the atrial cavity does not extend to the extremities of the body, there is posteriorly a solid mass of undivided plerome in which part of the stomach and testis are embedded, and anteriorly the plerome is undivided and is produced into six muscular processes which are the six circumoral arms.

The bulk of both the ecto- and endoplerome, as well as the anterior and posterior undivided portions of the plerome, is made up of a form of connective tissue consisting of a structureless or sometimes slightly fibrillated matrix in which a few corpuscles are embedded. This matrix stains, like all intercellular substance, with picro-nigrosin and picro-indigo-carmine, and its characters are fairly well shown in figs. 22, 26 and 30. The connective-tissue matrix is honeycombed with irregular cavities, and the muscles and all the principal organs of the body are, as it were, embedded in it. The blood-spaces have no regular arrangement, and I could not find any trace of definite blood-vessels such as occur in most simple Ascidians, unless a delicate pavement epithelium lining some of the larger cavities near the heart might be taken as an indication of regular blood-channels.

Musculature.

All the muscles of the animal are contained in the ectoplerome or in the undivided plerome of the anterior end. There is no trace of a layer of muscle-fibres surrounding the gut or the branchial sac. The general arrangement of the muscles is shown in figs. 3 and 4. The musculature is weak, except in the regions of the atrial and branchial siphons and the arms. Over the greater part of the body it consists of a series of thin longitudinal bands, placed rather far apart, and these are crossed at right angles by a series of circular bands, the two series forming a rectangular network with rather open meshes. The arrangement of these muscle-bands in Oligotrema is exceptional in that the circular bands are external and the longitudinal bands internal.

Towards the posterior end of the body the musculature diminishes and eventually dies out altogether, but it is strongly developed round the atrial siphon. The last-named structure is a long, narrow tube formed by the walls of the ectoplerome, and in a surface view, such as is shown in fig. 3, it might easily be taken for a continuation of the intestine. The muscles in this siphonal tube are arranged, like those of the body-wall, as an external layer of circular and an internal layer of longitudinal muscle-bands, which here are relatively very numerous and form a rectangular network, with very fine meshes.

Immediately below the branchial aperture the circular muscle-bands are gathered together to form a strong sphincter surrounding the mouth. This branchial sphincter (fig. 4, *sph.*) lies above a ring of thickened tissue surrounding the mouth, and embraces the branchial siphon. It is chiefly made up of a number of flat, concentric, muscular bands, which extend outwards over the bases of the tentacular arms in such a manner as to give a hexagonal figure to the sphincter. These circular muscle-bands are to be regarded as the constrictors of the branchial orifice, and they are crossed by a number of much finer radial bands or dilators of the branchial orifice, whose inner ends are inserted on the thickened ring of tissue mentioned above. The same ring of tissue serves for the attachment of the longitudinal muscles of the body-wall and the flexor muscles of the arms, to be described further on. The muscle-bands of the dilators, as well as those of the constrictors, branch, and the branches of the latter inosculate with one another in a complicated manner, especially at the bases of the arms (fig. 5, *sph.*). At the base of each arm a bundle of muscle-fibres is given off from the outermost band of the sphincter and branches freely in the substance of the arm, the branches looping round in the manner shown in fig. 5, and uniting again to form a number of bands which run back down the length of the arm and are inserted, like the dilators of the branchial orifice, on the ring of thickened tissue surrounding the mouth. One of these longitudinal bands, situated on the internal or adoral aspect of the arm, is especially large and thick (fig. 5, *mus. flex.*) and is to be regarded as the flexor muscle of the arm, bending it inwards towards the branchial orifice; while the slips derived from the outermost band of the sphincter would appear to act as extensors of the arm, lying as they do on its outer or aboral aspect. One may infer, from the arrangement of the muscles, that the arms in the living animal could be closed rapidly and powerfully over the branchial orifice, and would therefore be serviceable in capturing prey; but it should be noticed that the lateral offsets of the muscular core of each arm, which obviously fit into the pinnæ, contain no muscular slips. It must be concluded, therefore, that while the arm as a whole is movable, the pinnæ are incapable of independent movement.

The presence of muscular arms surrounding the branchial opening, though not unknown, is an unusual feature in Tunicata. The only Tunicate having arms comparable in size and complexity to those of Oligotrema is Octacnemus, and there is some resemblance in the arrangement of the muscles in these two forms. But the anatomy of Octacnemus differs so much in other respects from that of Oligotrema that there can be no question of close relationship, and one must look for a nearer homology to the six denticulate muscular lobes surrounding the branchial aperture of Ctenicella, which in turn are modifications of the six simple lobes characteristic of the branchial aperture of the Molgulidæ. Other Ascidians, of course, have similar lobes, but the number is not the same. The circumoral lobes of Ctenicella are described by de Lacaze Duthiers as muscular, and as having each one terminal and two pairs of lateral pinnæ. If they were largely increased in size, and their lateral pinnæ were multiplied and complicated by branching, and the circlet of lobes were somewhat further removed from the branchial aperture, the lobes of Ctenicella would bear a strong resemblance to the arms of Oligotrema. In the former genus the atrial aperture is also surrounded by denticulate lobes, four in number. These are not present in Oligotrema, but their absence is easily accounted for by the reduction of the atrial siphon. In my specimen the muscular branched lobes, a single one of which is shown in fig. 5, were so much shrunk by the action of spirit that they fitted loosely into the corresponding cavities of the test (fig. 4); but there can be no doubt that in life they fitted close into these cavities, and that each arm, though covered by a tough coat of test, was capable of a considerable amount of movement. It should be observed that the muscles are not continued into the lateral processes which must have fitted into the pinnæ, and it would therefore appear that while the arm could move as a whole, the pinnæ were incapable of independent movement. Every muscle-band is made up of a number of fairly long tapering muscle-fibres, each with a nucleus situated at about the middle of its length. The individual fibres exhibit a faint longitudinal striation and are crescentic in cross-section, the nucleus lying in the concavity of the crescent (fig. 29, *mus.*). I was unable to find any trace of undifferentiated protoplasm surrounding the nucleus.

The Atrium and Peribranchial Cavities.

The relations of the atrium and peribranchial cavities are best seen in the series of sections, figs. 8—15, and are also indicated in the diagram fig. 34, which is a reconstruction of my series of sections. The lumen of the atrial siphon is narrow (fig. 15, *at. si.*); internally it opens into the wide atrium or cloaca, *at.*, which lies, like a flattened sac, over the dorsal surface of the stomach, and extends right and left over about one third of its lateral surfaces. As explained above, the test is invaginated for a considerable distance at the atrial aperture, and forms a thin but perfectly distinct lining to the narrow atrial siphon, extending as far as the openings of the oviducts and the anus. Posteriorly the atrium is prolonged backwards on the right side of the body as far as the lowest limit of the bend of the intestine, but there is no corresponding cavity on the left side. Anteriorly the atrium extends forward between the stomach and the dorsal body-wall (ectoplerome) to about the middle of the length of the body, and is there interrupted by the dorsal suspensory fold (fig. 34, *D. sus.*); but laterally it extends right and left of the stomach and œsophagus towards the mid-ventral line, to form the right and left peribranchial cavities (fig. 13, *R. pbr.* and *L. pbr.*), separated from one another by the ventral suspensory fold. The peribranchial cavities are prolonged forward far beyond the limits of the atrium itself; as shown in fig. 12, they are completely separated from one another (except for their communication by way of the atrium) before they reach the level of the branchial sac. In the region of the branchial sac the peribranchial cavities continually diminish in size as they pass forwards (figs. 8 to 11), until in the most anterior branchial region they are reduced to mere canals, which lie dorsally right and left of the branchial sac, and eventually end blindly in front. The left peribranchial cavity is deeper dorso-ventrally, and extends rather further forward than the right. The limits of the right peribranchial cavity are indicated by the deeper shade of blue in fig. 34.

The walls of the atrium and peribranchial cavities, where they are not bulged by the gonads, intestine, and other viscera embedded in the ectoplerome, are for the most part smooth and devoid of ridges and folds. But in the œsophageal region a thick, longitudinal ridge of the ectoplerome, covered, of course, by atrial epithelium, projects into the peribranchial cavity of either side. As these ridges are highly vascular—that is to say, they contain an exceptional number of blood-spaces crowded with corpuscles,—they are probably respiratory in function, and I regard them as the representatives of the endocarps of other Ascidians.

The Branchial Siphon, Branchial Sac, and Gut.

The general relations of these organs and of the other viscera associated with them are shown in figs. 3 and 34, and in the series of sections, figs. 6 to 15. The branchial aperture has the form of a transversely elongated slit, and the branchial siphon, lined by a thick involution of the test, is also compressed in the transverse plane. The mouth at the bottom of the branchial siphon is not a circular orifice, but is narrow and slit-like, and slants dorso-ventrally owing to the branchial siphon being produced backwards on the ventral side to form a sort of half tube or gutter, crescentic in section. The mouth is surrounded by a circle of small, ramified, oral tentacles. I was unable to determine the number of these tentacles from my sections, but they are fairly numerous. I should judge that there are sixteen or more, and they are distinctly, but not largely, ramified. They are covered by a peculiar, modified, flagellate epithelium, which also lines the prebranchial zone and a large part of the branchial sac, and will be described in detail further on.

The branchial sac is one of the most remarkable features of Oligotrema, both because of its small size and because of the great reduction of the characteristic structures associated with it, such as the endostyle. The latter structure, indeed, is so feebly developed that no trace of it could be seen through the transparent walls of the body, even after the specimen had been stained with borax carmine and cleared in xylol preparatory to being embedded in paraffin. Under the same conditions the branchial sac itself could hardly be recognised, but it could be discovered, on close observation, as a dilatation of the anterior end of the œsophagus (fig. 3, *Br. s.*). Its extent and relations to the branchial siphon and adjacent organs are clearly shown in fig. 34. The œsophagus is a relatively wide tube running back on the dorsal side; its length is about one third of the whole length of the body, and it is therefore a much more important and conspicuous section of the alimentary canal than is the case in other simple Ascidians. Posteriorly its diameter gradually increases, and it passes without any obvious line of demarcation into the stomach. The stomach is flexed ventrally and has the form of a wide, U-shaped sac, occupying the posterior end of the body. The intestine takes its origin from the anterior end of the distal or ventral limb of the stomach. On leaving the stomach it passes into the ectoplerome of the left side of the body and runs backwards and towards the dorsal side; on reaching the dorsal side of the proximal limb of the stomach it turns forward and runs parallel with the œsophagus towards the atrial siphon. The anus is situated on a conical eminence in the atrium, opposite the atrial siphon. There is no trace of a digestive gland or liver, but the distal limb of the stomach is closely invested by the ramifications of the testis.

These organs may now be described in detail.

The Branchial Sac.—This organ is extremely small as compared with that of other Ascidians, its length being less than one fifth of the whole length of the body. Its relations are best understood by following a series of sections forward from the region of the œsophagus. As is shown in figs. 3 and 34, the œsophagus is a relatively long and wide tube lying on the dorsal side. Fig. 12 is a transverse section taken a short way below the posterior limit of the branchial sac. The œsophagus, invested by the endoplerome, is seen on the dorsal side attached by the thick and short dorsal suspensory fold to the body-wall. The ventral suspensory fold is long and thin, and separates the right and left peribranchial cavities from one another. Fig. 11 is a section through the extreme posterior limit of the branchial sac, and shows that here the ventral suspensory fold has become much thicker; and its ventral moiety is channelled by a few irregular passages which communicate with the right peribranchial cavity by elongated slit-like apertures or stigmata. A few sections further forward similar passages are found communicating by stigmata with the left peribranchial cavity, and still further forward the ventral suspensory fold is conspicuously thicker and contains a considerable central cavity, into which a number of irregular lateral chambers open. These in turn communicate by slit-shaped stigmata with the right and left peribranchial cavities. Fig. 10 is a drawing of a section taken still further forward, and shows that the cavity in the ventral suspensory fold—which cavity we may recognise henceforth as the branchial sac—has increased greatly in extent and communicates by a narrow passage with the œsophagus. The right peribranchial cavity retains its size, but the left cavity is notably diminished owing to the dorsal extension of the left corner of the branchial sac. Fig. 9 represents a section taken through the middle of the branchial sac. It shows that the ventral part of the branchial sac extends widely to the right and left, and that there is a corresponding reduction of the two peribranchial cavities. The cavity of the branchial sac communicates on each side by wider or narrower orifices with a number of secondary cavities, which may be called exhalent chambers. These open in turn by numerous stigmatic slits into the right and left peribranchial cavities. The œsophagus communicates freely with the branchial sac, but is still distinct from it, and, as will be shown directly, is lined by a different kind of epithelium.

Fig. 8 is a section through the upper end of the branchial sac. The peribranchial cavities are reduced to small dimensions; the cavity of the branchial sac has increased very much in size, and the œsophagus is reduced to a deep groove on its dorsal surface; but the epithelium lining the groove is still sharply marked off by its histological characters from that of the branchial sac proper.

Still further forward, at the extreme anterior end of the branchial sac, the peribranchial cavities are reduced to mere canals, into which a few stigmata open. The groove lined by œsophageal epithelium may be traced as far forward as the prebranchial zone, and there it dies out altogether.

It should be observed that the anterior extensions of the peribranchial cavities are pushed further and further towards the dorsal surface, so that the stigmata, which in the posterior part of the branchial sac were confined to its ventral and ventro-lateral aspects, are in the anterior part confined to its dorso-lateral aspects; and the branchial sac itself becomes progressively wider in its dorsal and narrower in its ventral moiety as one proceeds forwards. A little below the level of the branchial sphincter the ventral wall of the branchial sac is continued into the spout-shaped ventral prolongation of the branchial siphon, but the relations are somewhat complicated by the fact that in the mid-ventral line the branchial sac is produced into a short, forwardly directed diverticulum, lying ventrad of the branchial siphon. The walls of this diverticulum are thrown into longitudinal folds and are surrounded by the strong muscular fibres of the sphincter and the radial muscles taking their origin from the ring of thickened tissue round the mouth.

The result of these arrangements is that the obliquely directed mouth-opening faces the opening of the œsophagus into the branchial sac, and the stigmata are confined to a narrow zone running obliquely across from the ventral to the dorsal side of the branchial sac, nearly parallel to the mouth. These relations are clearly shown in the diagram, fig. 34. The space between the stigmata and the mouth is the prebranchial zone.

The branchial sac is too short and the stigmata are too closely crowded near its ventral surface to leave much room for an endostyle. Reference has already been made to the fact that in the entire animal, notwithstanding the transparency of its tissues, no endostyle could be seen; nevertheless one is present, though in a very reduced and rudimentary condition.

I had great difficulty in making out the relations of the endostyle, peribranchial grooves, etc., because the epithelial lining of the branchial sac was largely peeled off and lay in strips and patches in its cavity. In places, however, the epithelium remained adherent to the walls, and even in the detached strips the cell elements were so well preserved that I could study their details with some considerable degree of accuracy.

The œsophagus is lined by a glandular epithelium, the characters of which are shown in fig. 17. It is made up of columnar cells, with flattened nuclei situated at the extreme basal ends of the cells. The cytoplasm is filled with minute, highly refracting, yellowish granules, probably zymogen granules of some kind. This epithelium is continued up the groove on the dorsal side of the branchial sac, and may be traced nearly as far forward as the dorsal tubercle, beyond the point where the groove ceases to be recognisable.

De Lacaze Duthiers describes a groove which runs forward from the œsophageal opening alongside of the dorsal lamina in Molgula, with which the much larger œsophageal groove of Oligotrema is doubtless homologous; but I could find no trace of a definite dorsal lamina.

The epithelium lining the branchial sac and the prebranchial zone and covering the oral tentacles is of an entirely different character to that of the œsophageal groove, being composed of elongated cells such as are figured in fig. 18, *a*, *b*, *c*. They are extremely like the collar-cells of sponges, but as I was unable, after long examination, to satisfy myself that their long projecting free ends are, in fact, hollow cylinders with a flagellum passing down the middle of each, I will simply call them modified flagellate cells. These cells, as seen in section, are hammer-shaped; the head of the hammer is basal, and the nucleus is situated where the handle is fixed on the head. Adjoining cells are fitted together by their hammer-headed basal ends, and their handles project as long, free processes into the branchial or prebranchial cavities. The body of the cell (i. e. the head of the hammer) is more granular than the process, and stains with carmine; the process is stained, but not deeply, by picro-nigrosin or picro-indigo-carmine. In some places the free processes are short (fig. 18, *c*); in others they are inordinately long (fig. 18, *b*). In a considerable number of the cells I was able to detect a single delicate flagellum projecting from the free extremity of each; these flagella were most often recognisable in cells from the epithelium of the tentacles. In the majority of cells, however, no flagellum could be detected, and in many cases, especially in those cells in which they were longest, the free processes are club-shaped, having rounded extremities attached to the body of the cell by a relatively narrow pedicle. This feature suggests that the cells, like the endoderm-cells of Hydra, may withdraw their flagella and become amœboid. Fig. 18, *b*, is a careful camera drawing of some of the best preserved of these cells, very highly magnified. The resemblance to a collar-cell is certainly very striking, especially in the tallest cell on the right hand of the figure; but it would be rash to make any positive assertion about it. It must be remembered that my single specimen of Oligotrema was preserved in the tropics far from a laboratory, and that it had been in spirit for a long time before it reached me. It was therefore very unlikely that the details of such delicate structures as collar-cells could be faithfully preserved, and it says much for Dr. Willey's skill and care in preserving his collections that the specimen reached me in such good histological condition as it is. Though I have looked through sections of various Ascidians belonging to the genera Molgula, Ascidia, Phallusia, Ciona, and Styelopsis, I have been unable to find any trace of an epithelium comparable with that of the branchial sac of Oligotrema, and, so far as I can ascertain, no such epithelium has been described in any other Ascidian. The nearest approach to it that I can find is a band of elongated claviform cells, described by R. Hertwig as occurring in the ventral groove (Bauchrinne) of Cynthia canopus. In some places, where they are not well preserved, the cells of Oligotrema resemble those figured by him; but his drawings show the nuclei near the free ends of the cells, whereas in Oligotrema they are situated at the basal ends.

This modified epithelium clothes the tentacles and lines nearly the whole of the branchial sac, but does not occur in the œsophageal groove. There are also tracts in the branchial sac where it passes into a layer of simple non-flagellated cubical cells, and these in turn are succeeded by a tract of wedge-shaped, ciliated cells, as shown in fig. 18, *c*. A short band of these ciliated cells could be traced on the ventral side of the branchial sac, just in front of the branchial slits, and I regard this band as the representative of the endostyle. I cannot be certain of its extent, as the epithelium is largely peeled off in this region and lies in strips in the branchia sac. A similar band of ciliated epithelium can be traced on either side of the branchial sac, apparently nearly coinciding with the anterior limits of the right and left peribranchial cavities; but the condition of the epithelium prevents my saying anything certain on this point. At all events, the lateral bands of ciliated epithelium exist and run from the ventral towards the dorsal surface on either side of the branchial sac. They must therefore be regarded as representing the peribranchial grooves.

At the lips of the œsophageal groove, where the epithelium is marked with a black line in figs. 9 and 10, there is on either side a band of richly ciliated epithelium, which runs all along the lips of the groove and passes rather suddenly on their ventral side into the characteristic flagellated epithelium of the branchial sac. These bands of ciliated epithelium are very fairly well preserved; their component cells are represented in fig. 20, and it is obvious that they differ from the ciliated cells of the endostyle and peribranchial grooves, and belong rather to the category of the œsophageal epithelium. Dorsally these ciliated bands are succeeded by a tract of non-glandular cubical cells, which pass into the glandular epithelium of the œsophageal groove. These ciliated tracts may possibly represent the dorsal lamina. Their function is clearly to direct food into the œsophagus.

The Branchial Slits.—The branchial sac, as has been described, commences posteriorly as an irregular space in the thickness of the ventral suspensory fold. The thick lateral walls of the sac are honeycombed by a number of irregular canals and chambers, which recall, more than anything else, the incurrent and excurrent canals of a sponge. The chambers open on the one hand by wider or narrower orifices into the cavity of the branchial sac, and on the other hand they communicate by means of numerous stigmata with the peribranchial cavities. The arrangement of the chambers and stigmata is so complicated that it is difficult either to figure or describe it, but some idea of their relations may be gained by a study of fig. 16, which represents the portion of the branchial wall marked *z* in fig. 9, as seen from the inner or branchial surface. The drawing is made from a model reconstructed from about thirty serial sections, and shows the lower moieties of the two incurrent branchial chambers separated by a stout vertical fold of the branchial wall; this fold probably corresponding to one of the folds in the branchial sac of Molgula, while the incurrent chamber is a highly modified form of a cavity of a fold. Each chamber is subdivided in a most complicated way by cross-partitions and trabeculæ into a number of secondary chambers (fig. 26, *ic.*^{2}), which are prolonged upwards and downwards far beyond the limits of the openings into the primary incurrent chambers. Both primary and secondary incurrent chambers open by numerous elongate stigmata—some of which are clearly shown in fig. 16—into the peribranchial cavity, the walls of which are in places so deeply infolded as almost to form a set of excurrent chambers into which the stigmata open. Some idea of the complexity of these folds and chambers may be gathered from fig. 26, which is a drawing of one of the sections included in the middle of the model. The stigmata are fairly numerous and for the most part irregularly arranged, but they tend to rise one above another in a scalariform manner, as shown on the left hand of fig. 16. There is no trace of a spiral arrangement. The right-hand incurrent chamber of the model has ten stigmata opening from it, and the sections above and below the limits of the model show that about as many more open into the peribranchial cavity from its anterior and posterior prolongations.

The edges of the stigmata are clothed with a ciliated epithelium so similar to that of other Ascidians as to require no description.

The Œsophagus and Stomach.

The walls of both the œsophagus and stomach are longitudinally folded, but as the epithelium is more or less detached from the walls of the gut it is difficult to say how far the folds shown in figs. 12, 13, and 14 are natural, and how far they are due to contraction in spirit. The corresponding folds of the endoplerome in the stomach (fig. 14) indicate that in this region, at any rate, they are natural. The anterior section of the œsophagus is lined by a glandular epithelium exactly similar to that of the œsophageal groove; towards its posterior end the glandular cells increase in size and their nuclei become more rounded, and they gradually pass, without any definite line of demarcation, into the much longer and larger epithelial cells of the stomach, shown in fig. 27. The epithelium of the stomach is composed of very large wedge-shaped cells, with narrower basal and broader outer ends. The nucleus is situated near the basal end of each cell, is spherical, and has a distinct nucleolus. The cytoplasm of the narrower basal end is dense and finely granular, that of the broad outer end (i. e. the end turned towards the lumen of the gut) is vacuolated, and in most cases contains accretions of yellowish crystals, which are apparently products of digestion; but as I was unable to apply any micro-chemical tests to my sections I cannot say whether they are nitrogenous or not.

The form and relative size and extent of stomach and œsophagus are clearly shown in the diagram, fig. 34. The stomach exhibits a sharp ventral flexure, and is therefore U-shaped; the walls of the distal limb are not longitudinally folded, but are lined by the same kind of epithelium as those of the proximal limb.

The œsophagus of my specimen was empty, but both limbs of the stomach were full of material, including an entire Crustacean, which occupied nearly the whole of the cavity of the proximal limb. As the contents of the stomach serve to throw light on the habits of the animal, I examined them carefully and found that the smaller stuff and débris in the distal limb consisted chiefly of the fragments of Crustacea of different kinds; I could recognise fragments of the limbs and mouth parts and portions of the bodies of Copepods, some of which must have been of large size relatively to the specimen of Oligotrema in which they occurred. The entire Crustacean in the proximal limb proved to be an Amphipod which had evidently been only recently swallowed, for it is in a good state of preservation, the head and some of the anterior thoracic somites being the only regions in which signs of partial digestion are evident. As this Amphipod was cut into numerous sections I could not even determine its genus with any certainty, but from what I could make out of the shape of its antennæ, mouth parts, and thoracic limbs, from its dome-shaped thoracic segments, its long, backwardly directed and pointed thoracic pleura, and the shape of the abdomen, the terminal segments of which are fused together, and from the large size of the eyes (which, however, had suffered partial digestion), I shall not be far wrong in identifying it as a member of the Platyscelidæ, and very probably it is Platyscelus (Eutyphis) armatus, Claus, or a closely allied species.

Platyscelus armatus was taken by the "Challenger" in surface collections made between New Guinea and Japan, and P. rissoinæ was obtained by the same expedition at a depth of 240 fathoms off Tongatabu. It is not unlikely, then, that this Amphipod belongs to a genus of which members have been found swimming on the surface or at considerable depths in the Pacific. At all events there is no question here of a parasitic or commensal Crustacean, such as are found in the branchial sacs of so many Ascidians. The fragments of Copepods found in the stomach are obviously the digested remains of free-living species; the Amphipod belongs in all probability to the genus Platyscelus, the members of which are free living. Therefore the contents of the stomach go to prove that Oligotrema is able to capture and swallow relatively large and active Crustacea, a feat which its six muscular arms are well adapted to perform.

The intestine is a narrow tube which takes its origin from the left side of the anterior and dorsal angle of the distal limb of the stomach. It passes at once into the ectoplerome of the left side and runs backward, on the inner side of the ovary, towards the dorsal surface. On reaching the dorsal surface just in front of the posterior limit of the stomach it turns forward, and, still enclosed in a thickening of the ectoplerome, it is continued forward to the atrial siphon, and opens by a minute anal orifice in close proximity to it. The intestine of my specimen is empty. In the first part of its course it is lined by a glandular epithelium composed of small cells having the character, as far as their state of preservation allowed me to determine it, of somewhat squat goblet-cells. In the last section of the intestine the glandular cells give place to a columnar epithelium which appears to have an excretory function, as the free ends of its component cells are loaded with highly refracting nodules of a brownish-yellow colour (fig. 31), and a number of similar concretions are contained in the lumen. The blood-spaces surrounding this terminal section of the intestine are full of corpuscles of various kinds, whose accumulation in this region may be held to afford additional evidence of the excretory nature of the epithelium.

The Reproductive Organs.

Oligotrema, like other Ascidians, is monœcious. The ovaries are paired, and open into the atrial cavity near and somewhat in front of the anus, by right and left oviducts. The ovaries, as shown in figs. 3, 34, 13, 14, have the form of a pair of sinuous bands embedded in the ectoplerome of either side of the body at about the level of the atrial siphon. They are full of ova in all stages of maturation. The riper ova are relatively large, are full of large yolk-granules, and are enclosed in follicles and furnished with reserve cells after the manner common in Ascidians. The dorsal ends of the two ovaries are continued into short oviducts, which run in the ectoplerome and open into the atrial cavity at the ends of two papilliform prominences situated a short distance in front of the anus opposite the entrance of the atrial siphon (*R. od.*, *L. od.*, figs. 34, 14, 21). The terminal sections of the oviducts are lined by a ciliated cubical epithelium.

The testes are a mass of ramified tubules closely applied to ventral and ventro-lateral surfaces of the distal limb of the stomach. There is no external indication of their being paired, nor can any sign of paired structure be discovered in sections of these organs; but the sperm-ducts are paired and open at the ends of rather conspicuous nipple-shaped prominences into the right and left peribranchial cavities close to the angle of flexure of the stomach. Fig. 22 is a representation of the left spermiducal opening with an adjacent lobe of the testis, and incidentally illustrates the structure of the gastric and intestinal epithelium and of the connective tissue of the ectoplerome. In the testicular tubules one can distinguish externally a peripheral mass of larger rounded or polygonal cells with conspicuous and deeply stained nuclei; these I take to be spermatogonia; within these a layer of smaller cells, which seem to be spermatocytes, and in the centre a mass of developing spermatids, and in some of the tubules bundles of filiform spermatozoa. The cellular elements are minute, and the organ was too much macerated to admit of detailed study.

The Blood-vascular System.

I found traces of the heart in the form of a thin-walled, muscular tube, lying in the angle between the posterior end of the renal organ and the lobes of the testis; its position is indicated at *Ht.* in fig. 34. The muscular tube is surrounded by a distinct thin-walled sac, the pericardium, but as my sections of this region were broken by grains of sand carried through the preparation from the stomach on the edge of the razor, I was unable to make out the relations of the heart clearly, and could not determine the connection between the heart and neighbouring blood-spaces. Definite blood-channels can hardly be said to exist. The substance of the plerome is honeycombed by numerous cavities containing corpuscles, and in the neighbourhood of the heart a very delicate pavement epithelium could be detected forming a lining to some of the larger spaces. In each tentacular arm, however, there is a definite axial blood-space, which is enlarged and forms a conspicuous cavity at the base of the arm (fig. 6, *bl.*)

As a rule the corpuscles contained in the blood-spaces are few in number, but in some places they are very numerous, e. g. in the tissues at the bases of the arms, in the neighbourhood of the anus, in some of the endopleromic cavities bordering on the gut, and in the two longitudinal ridges which I have identified as endocarps. These blood-corpuscles may more appropriately be called amœbocytes. There appear to be two kinds—

1. A vesicular variety which are either (*a*) empty, when they have the form of nearly spherical vesicles (fig. 32, *c*, *d*) with a coarsely reticulated cytoplasm and a nucleus situated on one side of the cell; or (*b*) with cell contents, generally of a brown colour, which are sometimes arranged like a shell round the periphery of the cell (fig. 32, *e*), or are simply an aggregate of discrete granules (fig. 28, *am. c.*).

2. Granular basophile cells, such as those depicted in figs. 32, *a* and *b*, containing numerous granules which stain intensely with picro-nigrosin or picro-indigo-carmine. The granules can often be shown to be connected by fine threads, as is shown in fig. 32, *a*.

There can be little doubt that both kinds of cells are nutritive in function. As the vesicular cells are abundant in the blood-spaces surrounding the stomach, and their brown contents are of the same colour as the crystalline concretions which occur in such abundance in the epithelium of the stomach, I regard them as nutritive cells or trophocytes. The basophile cells are possibly reserve cells or thesocytes, but as I was unable to apply micro-chemical tests to my single series of sections I could not determine the nature of their contents.

The Renal Organ.

This organ is of very large size in Oligotrema, and lies embedded in the ectoplerome on the right and ventral side of the gut in the position shown in figs. 34, 14, and 15. It is somewhat ovoid in shape, with a broader anterior and a narrower and tapering posterior end. Briefly described, it is a closed, thin-walled sac, lined by a peculiar epithelium, and its cavity is filled with a mass of formless coagulum, the superficial layers of which are stratified and give rise to numerous spherical, concentrically striated concretions. There are no ducts and no communications of any kind between the cavity of the organ and the adjacent blood-spaces, but the anterior two-thirds of the sac bulge into the right peribranchial cavity, and are separated from the latter by so thin a wall that the contents might easily escape by diffusion into the peribranchial cavity, and so by the atrium to the exterior. A closed renal sac of this kind, filled with concretions, is universal in the family Molgulidæ, and has been described by de Lacaze Duthiers and Kuppfer; and a similar organ has been described by Van Beneden, Krohn, and others in various species of Ascidia and Phallusia. In Oligotrema the renal sac is exceptionally large, and is lined by an epithelium different from those described by the above-mentioned authors. A portion of this epithelium is represented in fig. 23. It consists of a layer of dark and finely alveolar protoplasm, in which rather large, rounded nuclei are embedded at intervals; there is no trace of cell outlines. This epithelium had been peeled off from the greater part of the inner surface of the renal sac, and the fragments were much shredded; it was only here and there that I was able to find adherent patches such as that represented in the figure. The strips lying loose in the cavity are very numerous, and those cut through in any section would go several times round the cavity if placed end to end—a fact which suggests considerable complication in the way of folds. The spherical concretions are often found embedded in the epithelium, as shown in fig. 24.

De Lacaze Duthiers, Kuppfer, and Krohn made a careful study of the contents of the renal sacs of the species they had under observation, and agree in stating that the spherical concretions gave the murexide reaction with nitric acid and ammonia; but in each case the reaction appears to have been faint, and their descriptions are not very convincing as to the presence of uric acid. In order to test this point I dismounted one of my slides and applied the murexide test, without obtaining any trace of a red or purple colour. The concretions and the surface layers of the coagulum were stained yellow, which suggested the xanthroproteic reaction and would seem to indicate the presence of proteid reserve material. It is obviously impossible to draw conclusions from chemical tests applied to sections which had previously undergone prolonged treatment for staining and mounting, but it is worth recording the fact that I failed to obtain the murexide reaction, for the structure and relations of this so-called renal sac are more suggestive of storage of reserve material than of an excretory organ, and the chemical composition of the concentrically striated spherules requires further investigation. In the spherules of Oligotrema the darker concentric bands were stained orange-red by borax carmine, and were deeply stained by hæmatoxylin. The central coagulum was unaffected by these dyes, but stained vividly with eosin; the outer stratified layers stained in the same manner as the spherules. Fig. 33 shows the manner in which the spherules seem to be formed from the external stratified layers of the coagulum contained in the renal sac.

The Nervous System.

The nerve-ganglion of Oligotrema occupies the position typical in Ascidians. It was not sufficiently well preserved to admit of thorough histological examination, but there is an external layer of rather large multipolar nerve-ganglion cells surrounding a central plexus of nerve-fibres. Anteriorly three stout nerves are given off from the ganglion, one of which passes straight forward to supply the dorsal part of the sphincter and the dorsal arms. The two other nerves pass right and left of the branchial siphon and supply the lateral and ventral parts of the sphincter and the lateral and ventral arms. Posteriorly a single nerve can be traced backwards for some distance in the mid-ventral line, and smaller branches appear to be distributed to the œsophagus.

The Subneural Gland and Duct.

The subneural gland itself was not well preserved. It appears to consist of a few short tubules closely applied to the nerve-ganglion. The interior of the tubules is occupied by numerous small cells with deeply stained nuclei, but the histology of the gland could not be satisfactorily determined. The duct, on the other hand, is large, and the tall, columnar, ciliated cells forming its epithelium were admirably preserved. The duct is short, and opens by a gaping slit-shaped orifice in the mid-dorsal line of the prebranchial zone. The orifice is situated on a small eminence, the dorsal tubercle, and, as far as could be determined from the sections, it is a simple longitudinal slit; there is no evidence of the spiral twisting of its lips so commonly found in other Ascidians.

In conclusion I must call attention to the cavities marked x in figs. 7 and 8. The cavity in fig. 8 is a posterior diverticulum of the larger cavity shown in fig. 7, and this is seen in the latter figure to be continuous with the space formed by contraction of the tissues between the spout-shaped ventral continuation of the branchial siphon and the body-wall. As the branchial siphon is an invagination of the test, the ectoderm is of course invaginated along with it, and as the siphon has shrunk away from the body-wall, the ectodermal epithelium remains adherent to the latter and forms a lining to it in the space just mentioned. This same ectodermic epithelium, characterised by the presence of black pigment granules, forms a complete lining to the cavity marked *x* and to its posterior diverticulum. It may further be seen that the cavity and the diverticulum are bounded by numerous strong, longitudinal muscle-bands, which, as seen in cross-section, appear to project into the cavities. The cavity with its diverticulum, shown in figs. 7 and 8, is on the left side; there is a similar cavity on the right side, with a much shorter diverticulum, but as the sections are oblique it does not appear in the figures. The ring of apparently thickened tissue lying below the branchial sphincter shown in fig. 4 is the external indication of the right cavity, and its walls are clearly corrugated by the contracted muscle-bands which traverse them. I am in doubt as to the nature of these cavities. They may, of course, be artefacts formed by deep infoldings of the ectoderm of the branchial siphon consequent upon the post-mortem contraction of the muscle-bands. But I am not inclined to interpret them as such, because of their symmetrical position right and left of the lower end of the branchial siphon and the upper part of the branchial sac. Nor can I imagine that any artificial folds due to post-mortem contractions could give rise to so well defined a diverticulum reaching so far back alongside of the branchial sac as that shown in fig. 8. Furthermore the connective tissue of the plerome adjacent to the cavities is differentiated, being far more compact and containing more numerous cellular elements than elsewhere, so as to give the appearance of a supporting structure related to the insertions of the longitudinal muscles. As it is impossible for me to form a satisfactory conclusion as to the nature of these cavities from the examination of a single series of sections, I must leave it an open question whether they are artefacts or not; but I am disposed to think that they are in some manner connected with the insertions of the muscle-bands both of the branchial sphincter and the longitudinal muscles of the body-wall, and that their thickened walls afford support to the tissues of this region.

It is clear from the foregoing description that Oligotrema, aberrant as it is in many structural features, must be classed in the family Molgulidæ, for the following reasons:

1. The arenaceous covering to the test and hollow, hair-like, villous processes of the latter, serving for the attachment of sand-grains, though found in some Cynthiadæ and other Ascidians, are features which on the whole are characteristic of the Molgulidæ.

2. The six branchial arms must be regarded as the highly differentiated representatives of six branchial lobes. The Molgulidæ are characterised by the possession of six branchial lobes, and in the genus Ctenicella, a member of this family, the lobes are produced into short, muscular, tentacle-like processes provided with terminal and lateral pinnæ comparable to the arms of Oligotrema, though very much less developed. The Molgulidæ are further characterised by the presence of four lobes surrounding the atrial siphon, and in Ctenicella these are modified in the same manner as the branchial lobes. There are no atrial lobes in Oligotrema, but their absence is clearly attributable to the great reduction of the atrial aperture.

3. The oral tentacles are compound, as is the case in all Molgulidæ.

4. The branchial sac is longitudinally folded, and the representatives of the longitudinal bars are not papillated.

5. The stigmata, though not arranged in spirals, are slightly curved. The stigmata of Oligotrema, indeed, do not show any marked resemblance to those of typical members of the family, but it must be remembered that the spiral arrangement does occur in all Molgulidæ.

6. The intestine is on the left side of the stomach, and throughout its course is embedded in the ectoplerome; or, to use the terminology of previous authors, it is attached to the

inner surface of the left side of the mantle, and is sunk in the mantle—a feature characteristic of Molgulidæ.

7. The renal organ is largely developed, is situated on the right side of the gut, and has the structure characteristic of the Molgulidæ.

8. The oviducts and sperm-ducts are paired, a feature which, though not universal in the Molgulidæ, is found in a great many members of the family.

But in classing Oligotrema in so well known and typical a family of simple Ascidians, its many peculiar features must not be lost sight of. These are—the wide separation of the branchial and atrial orifices; the great reduction in size of the atrial siphon and the concomitant suppression of the atrial lobes; the highly differentiated pinnate muscular arms; the great reduction in the size and extent of the branchial sac; the suppression of the dorsal lamina and the feeble development of the endostyle and peribranchial grooves; the relatively great length and diameter of the œsophagus, and the presence of a large œsophageal groove extending nearly to the anterior end of the dorsal side of the branchial sac.

It may be safely inferred, from what we know of the contents of the stomach of this single specimen, that these remarkable modifications of structure are due to changed habits of life, and particularly to a change from the mode of nutrition characteristic of other Ascidians. The large Amphipod contained in the stomach, whether I am right in referring it to the genus Platyscelus or not, clearly belongs to a free-living species, and is neither commensal nor parasitic. It is too large, and must have been too active, to have been swept into the mouth by the action of ciliary currents; and the same may be said of the relatively large Copepoda, of which abundant fragments are contained in the stomach. On the other hand, the size and position of the six tentacular arms, and the powerful and complex musculature with which they are provided, are satisfactory evidence of their efficiency as organs for capturing prey. Oligotrema psammites, then, is an Ascidian which captures and feeds on active

Crustacea of large size relatively to itself, and is no longer dependent on minute organisms and organic débris swept into its branchial chamber by ciliary currents and there filtered off from the water by an extensive branchial apparatus. Hence we find that the branchial sac, being no longer of prime importance for the nutrition of the animal, has undergone great reduction in size, as also have all those accessory organs whose function it is to secrete strings of mucus for the entanglement of the solid matter brought in by the branchial current and to direct the food by ciliary action along definite tracks to the œsophageal opening, viz. the endostyle, the peribranchial grooves, and the dorsal lamina. In consequence of the reduction in size of the branchial sac, and therefore of the stigmatic area, a relatively small current of water can pass through its meshes into the atrial cavity, and the reduction in size of the atrial siphon follows almost as a matter of course. On the other hand, the œsophagus and stomach are relatively far better developed than is the case in other simple Ascidians, and the dorsal œsophageal groove, facing the oblique elongated mouth-opening, becomes a prominent feature, being clearly adapted to transmit the organisms swallowed as food directly into the œsophagus.

In conclusion I must express my thanks to Professor W. A. Herdman, who was kind enough to read through my rough manuscript and to make many valuable suggestions, which I have incorporated in this paper; also to Professor Weldon, who, while I was working in his laboratory, assisted me from time to time in the solution of difficult problems of structure and histology. Most of all, my thanks are due to Dr. A. Willey, who, inadvertently perhaps, sent me the specimen of Oligotrema, which has been a subject of much interest to me.

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EXPLANATION OF PLATES 19—23,

Illustrating Dr. G. C. Bourne's paper on "Oligotrema psammites, a new Ascidian belonging to the family Molgulidæ."

Lettering in all the Figures.

amc. Amœbocytes. *an.* Anus. *arm.* Tentacular arm. *arm'.* Muscular core of tentacular arms. *At.* Atrium. *At. o.* Atrial aperture. *At. si.* Atrial siphon. *bl.* Blood-space. *Br.* Branchial aperture. *Br. s.* Branchial sac. *Br. si.* Branchial siphon. *d. sus.* Dorsal suspensory fold. *dt.* Dorsal tubercle. *ecp.* Ectoplerome. *enp.* Endoplerome. *ect.* Ectoderm. *ect. i.* Ectodermic ingrowth. *ex. c.* Excurrent chamber. *gl.* Subneural gland. *gr.* Yellow granules in ectoderm. *Ht.* Heart. *ic.* Incurrent chamber. *int.* Intestine. *l. mus.* Longitudinal muscles. *L. od.* Left oviduct. *L. pbc.* Left peribranchial cavity. *L. spd.* Left sperm-duct. *M.* Mouth. *mus.* Muscle-bands. *mus. dil.* Dilator muscle of branchial siphon. *mus. ext.* Extensor muscles of arm. *mus. flex.* Flexor muscle of arm. *N.* Nerve-ganglion. *n.* Nerve. *Oe.* Œsophagus. *œ. g.* Œsophageal groove. *Ov.* Ovary. *Od.* Oviduct. *pin.* Pinnæ of arms. *pl.* Plerome. *pbr.* Cavity of prebranchial zone. *R.* Renal organ. *R. od.* Right oviduct. *R. pbc.* Right peribranchial cavity. *R. spd.* Right sperm-duct. *Sph.* Sphincter muscle. *St.* Stomach. *St'.* Distal limb of stomach. *s. s.* Stigmata. *T.* Test. *tn.* Oral tentacles. *Tt.* Testis. *vp.* Vascular processes. *v. sus.* Ventral suspensory fold. *x.* Cavity (? artificial) lined by ectoderm. *y.* Bristle passed into the R. peribranchial cavity in fig. 34.

PLATE 19.

Fig. 1.—An enlarged drawing of O. psammites seen from the left side. The actual size of the animal is indicated by the line on the left of the figure.

Fig. 2.—An enlarged drawing of the oral aspect of O. psammites, showing the transversely elongated branchial aperture surrounded by six pinnate arms.

Fig. 3.—A view of the principal organs in situ, as seen through the transparent body-wall, after the test has been opened by a longitudinal cut.

Fig. 4.—A view of the branchial sphincter muscle and the six tentacular arms from below.

Fig. 5.—One of the muscular arms removed from the test, showing the arrangement of the muscles.

Fig. 6.—A section (somewhat oblique) through the branchial siphon and the bases of three of the tentacular arms.

PLATE 20.

Fig. 7.—A section through the mouth.

Fig. 8.—A section through the upper part of the branchial sac.

Fig. 9.—A section through the middle of the branchial sac.

Fig. 10.—A section through the lower end of the branchial sac.

Fig. 11.—A section through the upper end of the œsophagus, showing the thickening of the ventral suspensory fold, in which there are four small cavities, two of them opening by stigmata into the right peribranchial cavity.

Fig. 12.—A section through the middle of the œsophagus.

Fig. 13.—A section through the lower end of the œsophagus.

Fig. 14.—A section through the upper end of the stomach, showing the origin of the intestine from the ventral limb of the stomach and the openings of the oviducts.

Fig. 15.—A section passing through the atrial siphon and anus.

PLATE 21.

Fig. 16.—The inner surface of a portion of the wall of the branchial sac, showing the longitudinal branchial folds, the openings of two incurrent branchial chambers, and the stigmata opening into the latter. This drawing is made from a model reconstructed from a series of sections.

Fig. 17.—Cells from the glandular epithelium of the œsophagus.

Fig. 18—

a. Flagellate epithelium from the oral tentacles.

b.—Flagellate cells resembling collar-cells from the epithelium of the branchial sac.

c.—A portion of the epithelium of the branchial sac, showing the transition from the elongated flagellate cells to the ciliated epithelium of the endostyle.

Fig. 19.—Transitional epithelium from the œsophageal groove.

Fig. 20.—Ciliated cells from the lips of the œsophageal groove.

Fig. 21.—An enlarged drawing of the openings of the oviducts into the atrium.

Fig. 22.—Part of a section through the upper region of the stomach, highly magnified, showing the opening of the right sperm-duct.

Fig. 23.—A portion of the epithelium lining the cavity of the renal sac, highly magnified.

Fig. 24.—Two concentrically striated spherules from the renal sac, embedded in the renal epithelium (Zeiss $\frac{1}{12}$ hom. imm. comp., oc. 8).

Fig. 24*a*.—A double concentric spherule from the renal sac.

Fig. 25.—A magnified drawing of the area indicated by the reference number 25 in Fig. 6, showing the intrusive ectodermic tissue (*ec. i.*).

PLATE 22.

Fig. 26.—Part of a section through the walls of the branchial sac, highly magnified, showing the incurrent and excurrent branchial chambers and the stigmata. The drawing is made from one of the sections included in the model represented in Fig. 16.

Fig. 27.—Glandular cells from the epithelium of the stomach (Zeiss $\frac{1}{12}$ hom. imm. comp., oc. 4).

Fig. 28.—The opening of the duct of the subneural gland, highly magnified, showing the ciliated epithelium of the duct.

Fig. 29.—Outgrowths of the body-wall into the test, showing the pigmented ectodermic epithelium cells, many of which contain yellow granules (highly magnified).

Fig. 30.—A portion of the section represented in Fig. 25, very highly magnified, showing the intrusive ectodermal ingrowths (*ec. i.*) at the bases of the arms, and two vascular processes (*v. p.*).

Fig. 31.—A portion of the epithelium from the terminal part of the intestine, showing the excretory concretions.

Fig. 32.—*a* and *b*, basophile amœbocytes from the blood-spaces, highly magnified (Zeiss $\frac{1}{12}$ hom. imm., oc. 8); *c* and *d*, empty vesicular amœbocytes from the blood-spaces; *e*, a vesicular amœbocyte (trophocyte) with contents.

Fig. 33.—A portion of the stratified external layer of the contents of the renal sac, showing the formation of the concentrically striated spherules.

PLATE 23.

Fig. 34.—A diagram illustrating the anatomy of Oligotrema psammites. The tissues of the body-wall (ecto- and endopleromic) are represented in a light shade of blue, the gut in yellow, the test in pink, the ovaries in green, the renal organ and testis in different shades of red. The extent of the atrium and right peribranchial cavity is indicated by a darker shade of blue. The animal is represented as dissected from the right side, the right wall of the branchial sac being removed.